MAJOR ARTICLE

Human Herpesvirus 8 Infection within Families in Rural Tanzania

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Human herpesvirus 8 (HHV-8) infection is common in Africa. We examined the distribution of HHV-8 within families in rural Tanzania to determine routes of spread. HHV-8 infection was assessed by measuring antibody reactivity with a K8.1 (lytic-phase antigen) immunoassay. The prevalence increased from 3.7% (1/27) among infants to 58.1% (36/62) among children aged 3–4 years and 89.0% (65/73) among adults aged ≥45 years. Women with HHV-8–seropositive husbands had a 7-fold risk for infection (odds ratio [OR], 6.9; 95% confidence interval [CI], 1.9–25.3). HHV-8 seropositivity in children was associated with having at least 1 seropositive first-degree relative (OR, 14.7; 95% CI, 5.9–43.1), a seropositive mother (OR, 7.4; 95% CI, 3.2–16.8), a seropositive father (OR, 4.8; 95% CI, 2.3–10.1), or a seropositive next-older sibling (OR, 4.2; 95% CI, 1.9–9.4). Our data are consistent with the occurrence of HHV-8 transmission within families, from mothers and other relatives to children via nonsexual routes and between spouses via sexual routes.

Human herpesvirus 8 (HHV-8) is the etiologic agent of Kaposi sarcoma [1, 2]. Worldwide, the prevalence of HHV-8 mirrors that of Kaposi sarcoma [2] and is highest in sub-Saharan Africa. The reasons for the high prevalence in Africa are unknown. In the United States, high prevalence occurs in homosexual men, and infection is associated with number of sex partners, which suggests that sexual transmission occurs between men [3, 4]. Recent studies have also suggested that a small but significant transmission risk is associated with injection drug use [5, 6]. Evidence for heterosexual transmission is controversial. Modest associations with sexually transmitted diseases have been described among women in the United States [5, 6]. Two recent studies reported significant associations of HHV-8 seropositivity with multiple sex partners and with laboratory evidence of having had sexually transmitted diseases among adults in Nigeria and men in Kenya [7, 8]. A study of adults with cancer in South Africa found a statistically significant association between HHV-8 infection and reported numbers of sex partners [9]. In conflict with these studies, no evidence for sexual transmission of HHV-8 was found among heterosexual men and women attending a sexually transmitted diseases clinic in London, even among those who were born in Africa, in 2 other studies [10, 11].

Kaposi sarcoma has been described in young children who are not yet sexually active, particularly in Africa, which indicates that HHV-8 infection can occur during childhood [12–14]. Seroprevalences ranging from 20% to 50% have been reported among children who were

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Participants provided individual verbal consent, and parents provided verbal consent for the children to participate in serological surveys in Tanzania. Leftover archived samples from subjects in the original study were used for this study. Institutional review boards gave ethical approval for the study.

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not yet sexually active in sub-Saharan Africa [15, 16], in South America [17], and in Italy [18]. Significant correlations between the HHV-8 serostatus of mothers and children, between that of siblings, and between that of spouses were reported for Jewish families [19]. A study of people of African origin living in Guyana, South America, found similar correlations between the serostatus of children and their mothers and between that of siblings but not between the serostatus of spouses [20]. In the first family study to be done in Africa, we describe intrafamilial associations of HHV-8 in Tanzania, an area of very high HHV-8 endemicity.

SUBJECTS AND METHODS

Study population. The study population included residents of 9 rural villages in the North Mara District, near Lake Victoria, in northeastern Tanzania. The survey was conducted between May and June 1985 to study the prevalence of human immunodeficiency virus (HIV) infection in rural Tanzania. Because only 2 subjects were found to be infected with HIV, the samples were not analyzed further until the present study. The survey area consisted of fairly remote valleys and low hills. The population, which was largely Bantu, was engaged in subsistence farming. Villages were subdivided into administrative units of 10 households. A household was defined as an entity headed by a headman. In Tanzania, where polygamy was often practiced, headmen often had several wives in their households. Typically, a wife and her children lived in their own hut but would share a compound with other wives and their children, and other relatives of the headman might also live in the same compound. One compound per administrative unit was selected randomly, and all household members were invited by outreach survey staff to participate in the survey. We collected demographic information, including age, sex, marital status, and family relationships but not personal identifiers, and took a blood sample from each participant. Samples were available from all but 6 subjects (99.3% of the original study population) and were tested for HHV-8 antibodies.

Laboratory methods. We used 2 serological assays to determine HHV-8 infection. The primary assay was an ELISA using the K8.1 glycoprotein (a lytic-phase antigen), with serum diluted 1:20. This assay has been well validated in Africa and elsewhere [21, 22] and has been widely used in seroepidemiological studies. Although we have no data on whether titers decrease during storage, we believe that this is unlikely for antibodies in samples that have been maintained consistently at $-80°C$, as were the samples used in the present study. Furthermore, the high prevalence observed suggests that there was little loss in reactivity. The distribution of the optical density test results in our study subjects, although bimodal, was generally higher than that found in non-African populations, and some results were indeterminate. Therefore, we included an indeterminate zone to reflect our uncertainty about the exact cutoff value for positivity. Eighty-six subjects (10.8%) with readings in the indeterminate range were, therefore, excluded from the primary analysis. In separate analyses (data not shown), we found similar conclusions, regardless of whether the indeterminate results were classified as positive or negative.

We also tested the samples with a recently developed open-reading frame (ORF) 73 ELISA against the latency-associated nuclear antigen. The results of the ORF-73 assay have been found to closely correlate with the results of a standard immunofluorescence assay using latently infected cells (authors' unpublished data). This assay is still considered to be experimental, and therefore we present results based on the established K8.1 ELISA. However, the results of the ORF-73 assay support the validity of the K8.1 assay results ($\kappa = 0.63$), and the associations did not change when results from the ORF-73 assay, rather than the K8.1 assay, were used in the analysis.

Statistical analysis. To assess associations between the serostatus of family members, we computed odds ratios (ORs) and 95% confidence intervals (CIs) by fitting logistic regression models (PROC GENMOD in the SAS 8.0 software package; SAS Institute). In addition to the logistic regression model, we also used 2×2 tables and χ^2 tests to assess associations among binary variables (e.g., sex, elevation of village, and HHV-8 seropositivity). The first analysis assessed associations between the HHV-8 serostatus of spouses in this population. Because a husband often had >1 wife, we modeled a woman's serostatus as a function of her husband's serostatus and allowed for correlation among several wives. Second, we looked at parent-child associations, using the mother's and father's serostatus as covariates in the logistic regression model and allowing for correlations among siblings. Subjects <17 years old were considered to be children, and their ages were grouped into 3 categories: 0–4, 5–9, and 10–16 years. Third, we assessed associations between serostatus of siblings. Because children who are close in age have contact with each other more frequently than do children with greater age differences, we modeled a child's risk of being infected as a function of the infection status of the next-older sibling from whom we had a sample. Because having ≥ 1 sibling who is infected is dependent on sibship size, we also created a variable called "sib-ratio," which took values ranging from 0 to 1 and was defined as the proportion of HHV-8–infected siblings among siblings in the sibship. We used a χ^2 test for linear trend to examine the relationship between sib-ratio and the odds of being HHV-8 seropositive. We used generalized estimation equations to account for correlations between individuals in the same family [23]. We assumed equicorrelated working correlation matrices in the calculations

and that all members in a family have the same correlation. This is a reasonable assumption, because we included only spouses or first-degree relatives of the children in the analyses. Other working correlations yielded similar results. All analyses were adjusted for sex, child's age, and elevation of the village (hill or valley). Significance of association was estimated by use of the likelihood ratio test, and all P values were 2-sided.

RESULTS

Of 798 subjects, 357 (45%) were male, and 507 (64%) were <17 years old. The HHV-8 prevalence ranged from 54% to 93% in different villages. Figure 1 shows the distribution of HHV-8 serostatus as a function of age for male and female participants. HHV-8 seroprevalence increased steeply, from 3.7% (1/27) among children aged <1 year to 58.1% (36/62) among children aged 3–4 years, and continued to increase, although less steeply, throughout the age range we studied, reaching a peak of 89.0% (65/73) among individuals aged ≥45 years ($P < .001$; test for trend). The crude HHV-8 prevalence was higher among men than among women (88.4% vs. 79.0%; $P = .047$), but the difference was only marginally significant when we adjusted for age ($P = .076$). Women with HHV-8–seropositive husbands were more likely to be seropositive, even after we adjusted for the age of the women (OR, 6.9; 95% CI, 1.9–25.3). Participants residing in low-lying villages had a higher HHV-8 seroprevalence than did those residing in high-elevation villages (60.1% vs. 49.7%; $P = .04$).

Table 1 shows HHV-8 prevalence, according to the serostatus of first-degree relatives, among children stratified by age. Overall, the HHV-8 prevalence among children was 56.5% (247/437), ranging from 42.2% (65/154) among those aged 0–4 years to 67.7% (88/130) among those aged 10–16 years. In all 3 age strata analyzed, children with a seropositive first-degree relative (i.e., mother, father, or next-older sibling) had a consistently higher HHV-8 seroprevalence than did those with seronegative first-degree relatives. The effect of having a seropositive mother was greater than that of having a seropositive father or a seropositive next-older sibling. In analyses stratified by parent serostatus, children whose parents were both seropositive or whose mother was the seropositive parent had the highest HHV-8 prevalence (69.7% [62/89] and 70% [21/30], respectively), followed by those whose father but not mother was seropositive (45.2%; 42/93); the prevalence was lowest when both parents were seronegative (20.7%; 12/58). The differences in the HHV-8 seroprevalence among children with ≥1 seropositive relative and the prevalence among children with no seropositive relatives were most striking in the youngest age group. The prevalence ratio was 4.8-fold in the group aged 0–4 years, versus 2.7-fold in the group aged 10–17 years.

Table 2 shows ORs for the association of HHV-8 serostatus

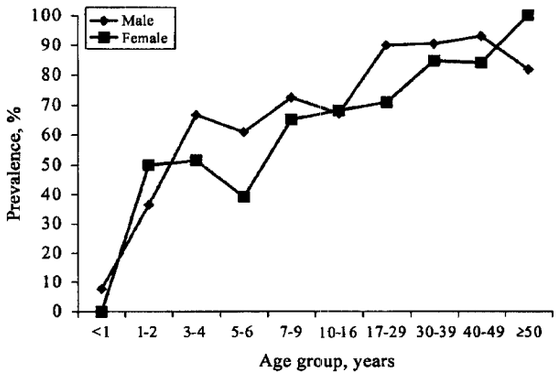

Figure 1. Age- and sex-specific human herpesvirus 8 prevalence in a study in rural Tanzania.

with selected demographic and relationship variables. In unadjusted analyses, HHV-8 seropositivity in children was associated with having ≥1 seropositive first-degree relative (62.8% for children with ≥1 seropositive first-degree relative vs. 13.8% for those with no seropositive first-degree relatives; OR, 14.7; 95% CI, 5.9–43.1); a seropositive mother (72.2% for children with seropositive mothers vs. 40.0% for children with seronegative mothers; OR, 7.4; 95% CI, 3.2–16.8); a seropositive father (61.4% for children with seropositive fathers vs. 37.3% for children with seronegative fathers; OR, 4.8; 95% CI, 2.3–10.1); and a seropositive next-older sibling (72.3% for children with a seropositive next-older sibling vs. 41.4% for children with a seronegative next-older sibling; OR, 4.2; 95% CI, 1.9–9.4). In multivariate analyses, compared with children aged 0–4 years, children aged 5–9 years had a 2.0-fold increase in risk of infection (95% CI, 0.9–4.6), whereas those aged ≥10 years had a 3.5-fold increase (95% CI, 1.3–9.5). HHV-8 seropositivity in a child was independently associated with having a seropositive mother (OR, 2.3; 95% CI, 1.0–5.2), a seropositive father (OR, 2.1; 95% CI, 1.0–4.5), and a seropositive older sibling (OR, 2.9; 95% CI, 1.3–6.7) in the multivariate analysis. In analyses of children with only 1 seropositive parent, those with a seropositive mother were more likely to be seropositive than were those with a seropositive father (70.0% vs. 50.6%, respectively; OR, 2.8; 95% CI, 1.1–7.8). In a stratified analysis, the effect of having a seropositive next-older sibling was significantly elevated in the stratum of seronegative mothers but not in the stratum of seropositive mothers (OR, 3.9, and 95% CI, 1.7–13.0, vs. OR, 1.1, and 95% CI, 0.3–3.9). Having ≥1 seropositive sibling predicted infection in the child (OR, 4.4; 95% CI, 2.0–10.0). This variable was dependent on sibship size. We also found a statistically significant trend between sib-ratio and the odds of being HHV-8 seropositive ($P = .002$).

Table 1. Frequency of human herpesvirus 8 (HHV-8) infection, by age group, among children in rural Tanzania, May through June 1985.

Characteristic	Children in age group, *n/N* (%)			
	0–4 years[a]	5–9 years	10–17 years	Total
Overall HHV-8 prevalence	65/154 (42.2)	94/153 (61.4)	88/130 (67.7)	247/437 (56.5)
Seropositivity in first-degree relative(s)				
≥1	36/75 (48.0)	60/85 (70.6)	46/66 (69.7)	142/226 (62.8)
0	2/20 (10.0)	1/19 (5.3)	5/19 (26.3)	8/58 (13.8)
Serostatus of relatives				
Mother seropositive[b]				
And older sibling[c] seropositive	21/25 (84.0)	13/17 (76.5)	7/8 (87.5)	41/50 (82.0)
And older sibling seronegative	9/14 (64.3)	7/10 (70.0)	2/3 (66.7)	18/27 (66.7)
Mother seronegative				
And older sibling seropositive	5/8 (62.5)	13/17 (76.5)	4/9 (44.4)	22/34 (64.7)
And older sibling seronegative	9/28 (32.1)	5/19 (26.3)	1/5 (20.0)	15/52 (28.8)
Both parents seropositive	20/35 (57.1)	23/31 (74.2)	19/23 (82.6)	62/89 (69.7)
Mother seropositive[d]	5/8 (62.5)	12/14 (85.7)	4/8 (50.0)	21/30 (70.0)
Father seropositive[d]	7/26 (26.9)	20/31 (64.5)	15/36 (41.7)	42/93 (45.2)
Neither seropositive	2/18 (11.1)	5/23 (21.7)	5/17 (29.4)	12/58 (20.7)

NOTE. *n/N*, no. of children with characteristic/total no. of children in group.

[a] Only 1 of 31 children aged <1 year was seropositive.

[b] Only children with ≥1 sibling were included in the analysis.

[c] "Older sibling" refers to the next-older sibling of the case child from whom a sample was available.

[d] Other parent seronegative.

DISCUSSION

We found significant correlations between the HHV-8 serostatus of spouses, parent and offspring pairs, and siblings in families residing in rural Tanzania. Women whose husbands were HHV-8 seropositive were 7-fold more likely to be infected. Children with a first-degree relative who was HHV-8 seropositive were also more likely to be seropositive. In a stratified analysis, children were 7 times as likely to be seropositive if the mother was seropositive and 4 times as likely if the father was seropositive. Having a next-older sibling who was seropositive was also associated with being seropositive among children whose mothers were HHV-8 seronegative. Taken together, our data imply that HHV-8 may be transmitted from parents to children and between siblings. In addition, there was a correlation between HHV-8 infection in spouses. This finding, coupled with the increase in HHV-8 prevalence with age observed in this and other populations [24], suggests that ongoing sexual transmission occurs during adulthood, although other explanations are possible [25]. The increase in HHV-8 prevalence with age in children who did not have a seropositive first-degree relative indicates that nonfamilial transmission also occurs.

The routes of HHV-8 transmission are unclear. Only 1 child of those aged <1 year was HHV-8 seropositive. This observation implies that maternal antibody levels in infants may be low and also that there was little or no vertical infection in this population. We propose that transmission to young children may occur through exposure to HHV-8–infected saliva, mainly from family members [24]. In sub-Saharan Africa, exposure to saliva may occur when mothers premasticate food for infants or clean children's faces with saliva. Children may also be exposed to saliva during play and sharing of eating implements with siblings, which probably results in transmission at older ages, as is thought to occur for Epstein-Barr virus [26, 27]. It has also been proposed that HHV-8 transmission through saliva occurs among homosexual men [25]. Like Epstein-Barr virus, HHV-8 is easily detected in saliva and pharyngeal tissue from homosexual men [24, 28], which provides ancillary support for the hypothesis that salivary transmission occurs between adults. The crowding, low socioeconomic status, and poor hygiene and sanitation that favor transmission of Epstein-Barr virus [29] may also facilitate HHV-8 transmission in sub-Saharan Africa. Direct sharing of saliva is, however, unusual among African adults. Kissing, a practice common in the West, is not usually practiced among rural Africans [30], although exposure to saliva among adults could occur through the sharing of utensils.

Whereas the high HHV-8 prevalence in sub-Saharan Africa and the cultural practices mentioned above may combine to increase the risk of transmission of HHV-8 in Africa above that in other areas, we note that HHV-8 transmission continues to occur frequently in older preadolescent children. The cultural practices that we postulated might expose these subjects to saliva are unlikely to provide a full explanation of the infection

Table 2. Frequency and risk of human herpesvirus 8 (HHV-8) infection among children aged 0–16 years in rural Tanzania, May through June 1985.

Characteristic	Frequency of infection, *n/N* (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Sex			
Male	125/213 (58.7)	1.0	1.0
Female	122/224 (54.5)	0.85 (0.5–1.4)	0.9 (0.4–1.9)
Age, years			
0–4	65/154 (42.2)	1.0	1.0
5–9	94/153 (61.4)	2.5 (1.4–4.6)	2.0 (0.9–4.6)
10–17	88/130 (67.7)	2.5 (1.3–4.8)	3.5 (1.3–9.5)
Serostatus of relatives			
Mother seronegative	66/165 (40.0)	1.0	1.0
Mother seropositive	104/144 (72.2)	7.4 (3.2–16.8)	2.3 (1.0–5.2)
Father seronegative	41/110 (37.3)	1.0	1.0
Father seropositive	124/202 (61.4)	4.8 (2.3–10.1)	2.1 (1.0–4.5)
Serostatus of older sibling[a]			
Seronegative	36/87 (41.4)	1.0	1.0
Seropositive	67/89 (72.3)	4.2 (1.9–9.4)	2.9 (1.3–6.7)
Elevation of village			
High	74/149 (49.7)	1.0	1.0
Low	173/288 (60.1)	1.6 (0.8–3.1)	2.3 (1.0–5.2)

NOTE. All variables were included in the multivariate model. CI, confidence interval; *n/N*, no. of children with characteristic/total no. of children in group; OR, odds ratio.

[a] "Older sibling" refers to the next-older sibling of the case child from whom a sample was available.

risk of these children, and they are not in a sexually active group. Future studies need to explore the risk factors for HHV-8 infection in this age group, using prospective data.

Our findings are similar to some of those reported by Plancoulaine et al. [20], but, in contrast to their study, we also found significant correlations between the HHV-8 serostatus of spouses and between the serostatus of a father and that of his children. In this regard, our study agrees with the recent report by Davidovici et al. [19] of HHV-8 serostatus correlation between spouses in Jewish families. Our study is the first to report on HHV-8 transmission within families in sub-Saharan Africa. We interpret the spousal associations as indicating heterosexual transmission, which supports the findings of 2 other studies of African populations, neither of which was family based [7, 8]. The correlation between the serostatus of a father and that of his children, even when the mother is seronegative, appears paradoxical, because fathers traditionally are not intimately involved in the care and upbringing of young children in east Africa. However, mothering responsibilities may be shared among wives and with other female relatives (paternal aunts and grandmothers) living in the compound. The infection status of these other relatives may correlate with the infection status of the father, thereby providing an alternative explanation for the association between the infection status of the father and that of his children when the birth mother is HHV-8 seronegative. We also observed a modestly significant but puzzling inverse association between HHV-8 serostatus and elevation of the village of residence that may be due to unknown environmental factors [31]. To summarize, our data imply that significant HHV-8 transmission risk exists within families. Transmission probably occurs through exposure to saliva generally and, among adults, through sexual transmission, but there may be other transmission routes as well.

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